NEUROTRANSMITTOR CHANGES IN THE RAT BRAIN AFTER PORTACAVAL ANASTOMOSIS

G. SIMERT, A. NOBIN[x], E. ROSENGREN[xx] AND J. VANG

From the Departments of Surgery, [x]Histology and [xx] Pharmacology
University of Lund, Lund (Sweden)

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ABSTRACT

Porta caval anastomosis in rats elevated the brain serotonin content by about 30%, the greatest increase being noted in the brain stem. A minor increase in norepinephrine content but no change in dopamine concentration was also noted. Low dose L-dopa treatment reversed the serotonin changes but did not affect catecholamine concentrations. Protein load tended to lower most transmittor concentrations without clinically affecting the animals.

The formation of 5-HT from tryptophan in vitro in slices of rat cerebral cortex was reduced by about 35% whereas the formation of 5-HIAA in the same slices was increased by about 60%. This in vitro study indicates that the portacaval shunt causes a change in the activity of serotonin neurons.

The findings suggest that changes in brain serotonin metabolism and brain serotonin neurons may be a cause of hepatic encephalopathy.

Patients with liver cirrhosis with and without a portacaval shunt (pc-shunt) often show hepatic encephalopathy, a syndrome consisting of acute and chronic neuropsychiatric symptoms commonly combined with hyperammonaemia. The cause of this cerebral dysfunction is not known. Pc-shunt in rats is often used as an experimental model to study this syndrome. The operation results in steady hyperammonaemia (26) and a neurological syndrome with pathological electroencephalogram (EEG) (12) and abnormal histological findings (7,8), all of which resemble changes seen in man with hepatic encephalopathy. Hepatic coma is however not possible to produce in rats with this experimental model.

The general expansion of interest in brain chemistry has led to several investigations on cerebral metabolic changes in rats after pc-shunt (9,16). One possible reason why hepatic encephalopathy develops would be altered turnover and concentration of transmittor substances in the brain. These alterations could conceivably be mediated through the changes in concentration of plasma amino acids, that are known to accur after pc-shunt (2) and in anhepatic animals (29). For example brain tryptophan and serotonin concentrations have been shown to be dependent on the plasma concentration certain amino acids (14,15). This investigation was undertaken to study the following problems concerning brain transmittor changes after portacaval shunts:(1). What changes in concentration of serotonin, norepinephrine, and dopamine in the rat brain can be produced by a pc-shunt? (2). Do regional differences in the changes in concentration of brain transmittors exist, indicating localized metabolic or functional changes in the rat brain after pc-shunt? (3). Can changes in brain transmittor concentrations after pc-shunt be reversed by treatment with L-dopa, a drug probably capable of arousing and individual from hepatic coma (1,18)? (4). What is the effect of protein given by mouth on the monoamine transmittor substances in rat brain? (5). To what extent does a pc-shunt

affect the rate of synthesis and storage of serotonin and 5-hydroxyindole acetic acid (5-HIAA) from labelled tryptophan in slices of rat cerebral cortex in vitro.

Material and methods

Adult (270-400 g) male rats (Wistar strain) were used. Four were placed in each cage and fed standard food pellets and water ad libitum. Sixty-two rats were subjected to pc-shunt as described by Lee (27), under light ether anaesthesia. Twenty-eight rats were operated including handling of the viscera but not the shunt procedure.

All animals were sacrificed by decapitation 6 weeks after operation. All decapitations took place between 10 a.m. and 3 p.m. All shunts were inspected and found patent at autopsy. In four animals post-mortem angiography via a mesenteric vein was made. All showed a patent shunt and no collaterals to the liver were seen. After decapitation the brain was quickly removed from the skull, the alfactory bulbs removed, and the brain dissected into telencephalon, diencephalon and brain stem for amine estimates (see Fig. 1); the cerebellum and pineal gland were discarded. The specimens were quickly frozen in liquid nitrogen and stored at this temperature until analysis could be done. Amine determinations were performed on pooled samples from 3 animals.

5-Hydroxytryptamine (5-HT, serotonin) was estimated spectrofluorometrically according to the method of Bertler (4). Norepinephrine (NE) and dopamine (DA) were determined according to Bertler, Carlsson, Rosengren, and Waldeck (5) as modified by Häggendal (22).

L-dopa treatment. Twenty-four animals with a pc-shunt were given 1.5 mg/100 g per kg body-weight. L-dopa intraperitoneally every day for seven days before killing. The last injection was given two hours before decapitation.

effect the rate of synthesis and storage of serotonin and 5-hydroxyindole acetic acid (5-HIAA) from labelled tryptophan in slices of rat cerebral cortex in vitro.

Material and methods.

Adult (220-400 g) male rats (Wistar strain) were used. Four were placed in each cage and fed standard food pellets and water ad libitum. Sixty-two rats were subjected to pc-shunt as described by Lee (27), under light ether anaesthesia. Twenty-eight rats were operated including handling of the viscera but not the shunt procedure.

All animals were sacrificed by decapitation 6 weeks after operation. All decapitations took place between 10 a.m. and 3 p.m. All shunts were inspected and found patent at autopsy. In four animals post-mortem angiography via a mesenteric vein was made. All showed a patent shunt and no collaterals to the liver were seen. After decapitation the brain was quickly removed from the skull, the olfactory bulb removed, and the brain dissected into telencephalon, diencephalon and brain stem for amine estimates (see Fig.1); the cerebellum and pineal gland were discarded. The specimens were quickly frozen in liquid nitrogen and stored at this temperature until analysis could be done. Amine determinations were performed on pooled samples from 3 animals.

5-Hydroxytryptamine (5-HT, serotonin) was estimated spectrofluorometrically according to the method of Bertler (4). Noradrenaline (NE) and dopamine (DA) were determined according to Bertler, Carlsson, Rosengren, and Waldeck (5) as modified by Hoggendal (22).

L-dopa treatment. Twenty-four animals with a pc-shunt were given 1.5 mg/100 g per kg body-weight. L-dopa intraperitoneally every day for seven days before killing. The last injection was given two hours before decapitation.

Protein load. Four animals with a pc-shunt and 4 sham-operated controls were given 10 ml of fresh rabbit blood via a gastric tube 5 hours prior to decapitation. In this experiment serotonin, norepinephrine, and dopamine were analysed in whole brain (cerebellum and pineal gland discarded).

Incubation with ^{3}H-tryptophan in vitro. The formation of 5-HT and 5-HIAA from ^{3}H-tryptophan in vitro in rat cortical slices was studied as described by Björklund, Axelsson and Falck (6). A brief account of the method follows. Thin rat cortical slices were incubated with ^{3}H-tryptophan (5 µCi/ml) for 30 min. After incubation the slices were homogenized in 400 µl 0.2 N acetic acid. Unlabelled carrier substances were added to the homogenate, and two 40 µl aliquots of the supernatant were spotted onto silica-gel thin-layer plates. 5-HT and 5-HIAA were separated from other metabolites by chromatography, scraped off the plate, and mixed with 1 ml water in the scintillation vial. The overall recovery was 50 % for 5-HT and 80 % for 5-HIAA.

In the incubation media ^{3}H-tryptophan was separated from the indole acids by passing the media through Sephadex G-10 columns. The eluate containing indole acids was freeze-dried, and the residue taken up with 200 µl 0.2 N NAc. Two 20 µl aliquots were spotted onto silica gel, and 5-HIAA was separated from other indole acids by chromatography. The average recovery of 5-HIAA was 100 %.

The readings were corrected for recovery. Blank values were obtained using identical tissue that had been boiled (20 min at 100°C) before incubation. Reported figures represent estimated values minus blanks.

Statistics. Owing to the pooling of cerebral regions from different rats, each figure represents a mean for three animals. The standard deviation and standard error of the mean must be assessed with this in mind. Individual variations between single rats may be greater than is apparent from our measurement of sprea-

ding. Differences in group means were tested by Student's t-test with $p = 0.01$ as the highest significance level.

Results

The experiments showed that 6 weeks after establishing a pc-shunt the content of serotonin in rat brain increased by about 30% (Table 1 and Fig. 2), which is highly significant. Minor differences in the increase in serotonin concentration were found in different regions: thus the increase was about 28% in telencephalon ($p < 0.001$), 20% in diencephalon ($p \geq 0.05$) and 35% in the brain stem ($p < 0.05$).

Fig. 3 shows the changes in cerebral norepinephrine 6 weeks after operation (see also Table 1). An increase of 10–15% took place in all regions and in whole brain, but these increases are significant only in brain stem ($p < 0.05$) and in whole brain ($p < 0.01$).

The changes in brain dopamine concentration after pc-shunt were small and not significant ($p \geq 0.05$) (fig. 4 and Table 1).

The rise in serotonin concentration caused by the portacaval anastomosis was completely reversed by L-dopa treatment (fig. 2). No change in norepinephrine concentration could be seen, and the increase in dopamine concentration was small and statistically insignificant (figs. 3 and 4; Table 1).

The animals in the protein load investigation were given 10 ml of rabbit blood 5 h before decapitation. The results of this experiment are shown in fig. 5 and Table 1. The protein load tended reduce most transmittor concentrations both in the controls and in the portacaval-shunted group. In the sham-operated controls statistical significance was reached only for dopamine, and in the pc-shunted group for serotonin and noradrenaline but not for dopamine. None of the rats in this experiment was clinically affected or showed behaviour anomalies.

The formation of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) from radioactive tryptophan in vitro in rat cortical slices was examined in pc-shunted rats six weeks after operation and in controls. The results are summarized in Table 2. The total amounts of 5-HT and 5-HIAA formed (calculated as the sum of the amines and acids in tissue and medium) were similar in shunted and control rats. Distinct differences in the dispersion of 5-HT and 5-HIAA formed were present, however: only 65 % of the amount of serotonin formed in control slices was synthesized in slices from shunted rats. In contrast in the pc-shunted rats the concentrations of 5-HIAA in both tissue and medium exceeded those in the control experiment by 150 % and 170 % respectively.

Discussion

In the first experiment in our series we demonstrated a rise in brain serotonin concentration after the establishment of pc-shunt. This is in accordance with previous investigations (3,9,10). Diurnal variations in brain serotonin concentrations have been reported to be within the same size range as those found in our investigation (30). We have abolished this source of error by sacrifying the animals at approximately the same time of day. Furthermore, preliminary results from our laboratory suggest that diurnal changes in brain serotonin concentrations in our rat strain are insignificant (Nobin et al., to be published).

A possible explanation of the changes in serotonin concentration is changed plasma amino acid concentration (14,15). Aquirre et al. (2) and Cummings et al. (9) have shown a characteristic pattern of changes in plasma amino acids 4 to 6 weeks after a portacaval shunt in rats and dogs. Thus plasma phenylalanine, tyrosine and histidine were significantly elevated 1 month after operation in rats while leucine, isoleucine and valine were all significantly decreased. The plasma tryptophan content showed no change (9). Similar changes in the amino-acid pattern have been reported in human liver cirrhosis (16,20).

Such changes in plasma amino-acid concentration induce increased concentration of brain tryptophan and serotonin.

This could be explained by the fact that tryptophan is competing with tyrosine, phenylalanine, leucine, isoleucine and valine for a common carrier system over the blood-brain barrier, and that the brain tryptophan and serotonin levels are correlated to the ratio of plasma tryptophan to the sum of the other five amino acids (15).

The rise in serotonin concentration could be reversed by L-dopa treatment, confirming earlier results (3). In this investigation we have chosen a scheme of administration and dose of L-dopa (1.5 mg/100 g b.w.) comparable to that suggested for treatment of chronic hepatic encephalopathy in man (28). This dose is small compared to that generally used in investigations of L-dopa effects on brain serotonin concentrations. It reduced the serotonin concentration without significantly changing the catecholamine concentration.

The elevation of brain serotonin concentration caused by pc-shunt was reversed by feeding the rats fresh blood through a gastric tube. In the control animals a smaller, insignificant reduction was observed. This is contrary to the theory that increased brain serotonin concentration could be a cause of hepatic encephalopathy as administration of protein generally makes hepatic encephalopathy worse in patients with this syndrome. In rats with pc-shunt, however, protein load does not aggravate symptoms of encephalopathy or change the state of consciousness or blood-ammonia levels (25). A possible explanation of the change in brain serotonin content is that a change in plasma-amino-acid pattern caused by the protein load tends to normalize brain serotonin levels. Another theory is that these changes constitute part of a stress reaction caused by handling and intubation of these animals. That this could be true is indicated by the general reduction in brain transmittor substance concentrations in our experiment. Further investigations are in progress.

The general changes in catecholamine concentration are less distinctive. The small but significant increase in norepinephrine concentration caused by pc-shunt is not affected by L-dopa treatment. A reduction after protein load corresponding to the fall in serotonin concentration took place. No significant changes in dopamine concentration could be found. Fisher (19) and Dodsworth (13), who performed experiments on comatose rats after ligation of the hepatic artery and pc-shunt noted low concentrations of norepinephrine. The two different experimental models for provoking a state similar to hepatic encephalopathy are not directly comparable. Our results do not support the hypothesis that hepatic encephalopathy is due to depletion of brain catecholamines and their replacement by false neurotransmittors (13,19).

Our in vitro study on the metabolism of labelled tryptophan in cortical slices has yieled interesting new data on the synthesis and release of 5-HT and 5-HIAA in brain tissue from pc-shunted rats compared to sham-operated controls. The total decarboxylating activity measured as the total amount of 5-HT and 5-HIAA formed is similar in both groups. The increased concentration of endogenous 5-HT in brain from pc-shunted rats could thus be a result of the increased availability of tryptophan in brain tissue (3).

The increment of 5-HIAA in tissue and medium from the pc-shunted rats is evidence of increased activity in the serotonin neurons especially at the synaptic level. This increased activity can also explain the reduced concentration of 5-HT in tissue slices from shunted rats. It is known that during increased impulsive activity in a neuron the newly synthesized transmittor is used first, implying that replenishment of the storage pool could be delayed.

The increased activity in the central neurons of the pc-shunted rats measured as the increased formation of 5-HIAA is comparable to the findings of Knell and collaborators (24) in patients with hepatic encephalopathy and of Baldessarini and Fischer (3) in pc-rats. Knell and collaborators (24) reported

that stupor or coma from fulminant hepatic failure was associated with high cerebrospinal fluid concentrations of 5-HIAA and also of the catecholamine metabolite homovanillic acid. Our in vitro study shows that the altered a indole metabolism in rat brain after establishing a pc-shunt is not only due to increased availability of the precursor, tryptophan, but is also dependent on a change in activity of the 5-HT-containing neurons.

Regional analysis of the serotonin concentration demonstrated that the increase differed in the three regions of the brain. Statistically significant increments were noted in the telencephalon and brain stem preparations, whereas no significant increase was measured in the diencephalon. These regional differences are in accordance with the findings of Curzon et al. (10) and Cummings et al. (9) both of whom reported that the greatest increase in serotonin concentration took place in the brain stem. It is of interest that the cell bodies of all central serotonin neurons are located to the brain stem (11).

The changes in concentration of catecholamines showed only minor regional differences. The only localized, significant increase was noted for norepinephrine in the brain stem.

Regional differences in rat brain following portacaval anastomosis have also been noted when measuring other parameters such as the concentrations of ammonia and carbohydrate substrate (21).

This investigation thus indicates that the syndrome of hepatic encephalopathy may be explained by changes in central serotonin neurons. Evidence of this is the increase in serotonin concentration that takes place after pc-shunt and its return to normal when L-dopa is given. The findings in vitro consitute further evidence for a profound change in the function of the serotonin neurons following portacaval anastomosis.

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Table 1.

Concentrations of serotonin, norepinephrine and dopamine in rat brain after different experimental procedures. Values are expressed in µg/g and give mean ± S.E.M.

Procedure	Serotonin				Norepinephrine			
	Brain stem	Dien-cephalon	Telen-cephalon	Whole brain	Brain stem	Dien-cephalon	Telen-cephalon	Whole brain
Sham operation	0.37±0.04	0.47±0.04	0.29±0.01	0.33±0.01	0.35±0.01	0.61±0.05	0.24±0.01	0.31±0.01
Portacaval shunt	0.50±0.01	0.56±0.01	0.37±0.01	0.42±0.01	0.40±0.01	0.69±0.02	0.26±0.01	0.35±0.01
Pc-shunt + L-dopa	0.37±0.01	0.45±0.01	0.29±0.01	0.33±0.01	0.38±0.01	0.62±0.02	0.29±0.02	0.35±0.01
Sham op + blood	-	-	-	0.30±0.01	-	-	-	0.25±0.02
Pc-shunt + blood	-	-	-	0.32±0.02	-	-	-	0.30±0.01

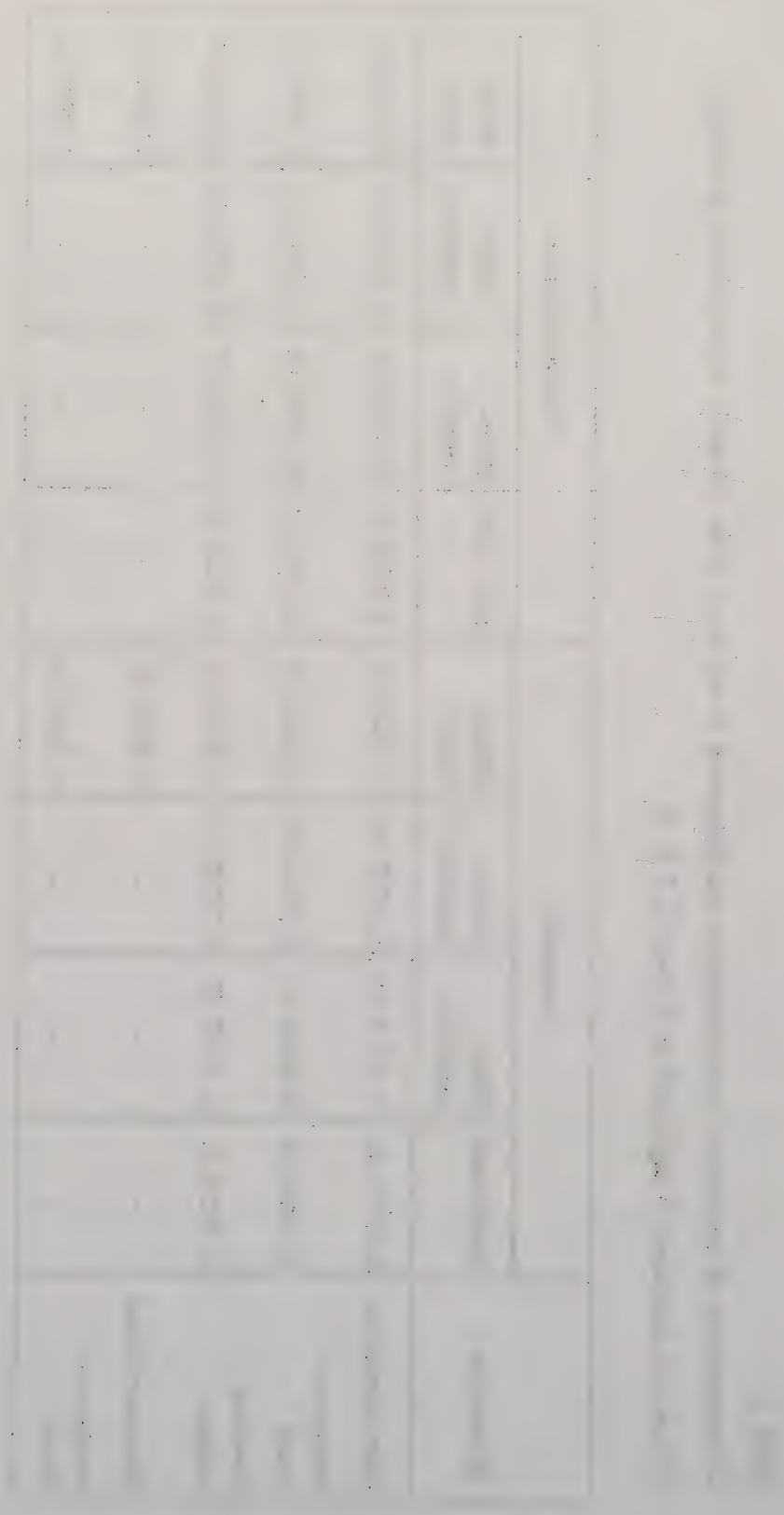


Table 1. (cont.)

Procedure	Dopamine			
	Brain stem	Dien-cephalon	Telen-cephalon	Whole brain
Sham operation	0.03	0.16±0.02	0.97±0.08	0.68±0.04
Portacaval shunt	0.025	0.17±0.02	1.08±0.04	0.71±0.02
Pc-shunt + L-dopa	0.04	0.19±0.02	1.11±0.07	0.77±0.05
Sham op + blood	-	-	-	0.51±0.06
Pc-shunt + blood	-	-	-	0.66±0.04

Table 2. Formation of 5-HT and 5-HIAA from ^{3}H-tryptophan in vitro in slices of rat cerebral cortex. Figures indicate cpm/mg tissue $\pm$ S.E.M. of 6 — 8 determinations. Student's t-test.

	5-HT in tissue	5-HIAA			5-HT + 5-HIAA total
		in tissue	in medium	total	
Control	245 ± 22	102 ± 18	105 ± 20	207 ± 28	452 ± 31
Pc-shunt	156 ± 13^{xxx}	152 ± 16^{xxx}	179 ± 24^{xxx}	331 ± 21^{xxx}	487 ± 22

Legends to figures

Fig. 1. Schematic representation of the dissection procedure. Projection of cutting lines on a near-mid-sagittal section of the rat brain. Telen, telencephalon; Dien, diencephalon; AC, anterior commissure; OC, optic chiasma; PC, posterior commissure.

Fig. 2. 5-HT-concentration in different brain regions and in whole brain six weeks after a portacaval shunt, with and without L-dopa treatment. L-dopa was given during the week before decapitation (see Material and Methods). For regional analysis preparations from three rats were pooled for each determination. The bars give percent of control ± S.E.M. of four determinations. The significances were calculated for differences between sham-operated and pc-shunted and pc-shunted + L-dopa-treated animals. Differences; $^{x} = 0.05 > p > 0.01$, $^{xx} = 0.01 > p > 0.001$, $^{xxx} = p < 0.001$. Student's t-test.

Fig. 3. NE-concentration in different brain regions and in whole brain six weeks after a portacaval shunt, with and without L-dopa treatment. L-dopa was given during the week before decapitation (see Material and Methods). For the regional analysis preparations from three rats were pooled for each determination. The bars give percent control ± S.E.M. of four determinations. The significances were calculated for differences between sham-operated and pc-shunted rats and between pc-shunted + L-dopa-treated animals. Differences: $^{x} = 0.05 > p > 0.01$, $^{xx} = 0.01 > p > 0.001$, $^{xxx} = p < 0.001$. Student's t-test.

Fig. 4. DA-concentrations in different brain regions and in whole brain six weeks after a portacaval shunt, with and without L-dopa treatment. L-dopa was given daily during the week before decapitation (see Material and Methods). For the regional analysis preparations from three rats were pooled for each determination. The bars give percent of control ± S.E.M. of four determinations. The significances were calculated for differences between sham-operated and

pc-shunted + L-dopa-treated animals. Differences: $^{x} = 0.05 > p > 0.01$, $^{xx} = 0.01 > p > 0.001$, $^{xxx} = p < 0.001$. Student's t-test.

Fig. 5. The concentrations of serotonin, norepinephrine and dopamine in whole rat brain after portacaval anastomosis and protein loading. The animals were killed 6 weeks after operation. The protein load consisted of 10 ml rabbit blood given via a gastric tube 5 h before killing. The bars give percent controls ± S.E.M. of four determinations. The significances were calculated within the sham-operated groups and within the pc-shunted groups. Differences compared to controls: $^{x} = 0.05 > p > 0.01$, $^{xx} = 0.01 > p > 0.001$, $^{xxx} = p < 0.001$.

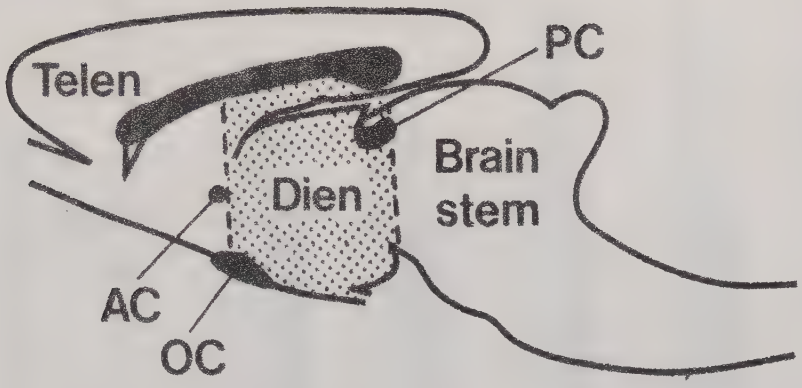
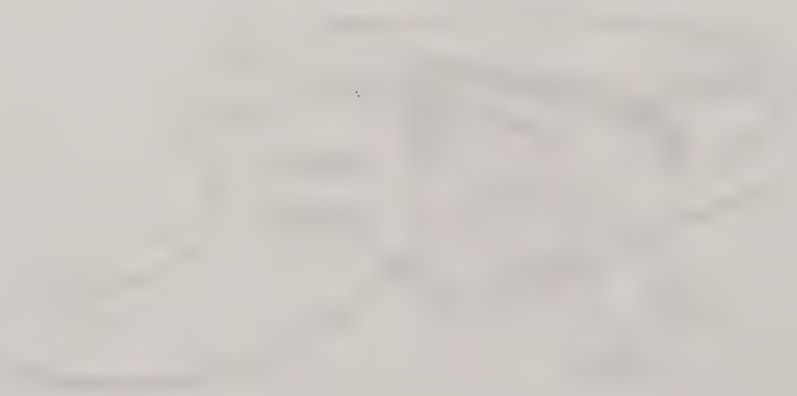


FIGURE 1



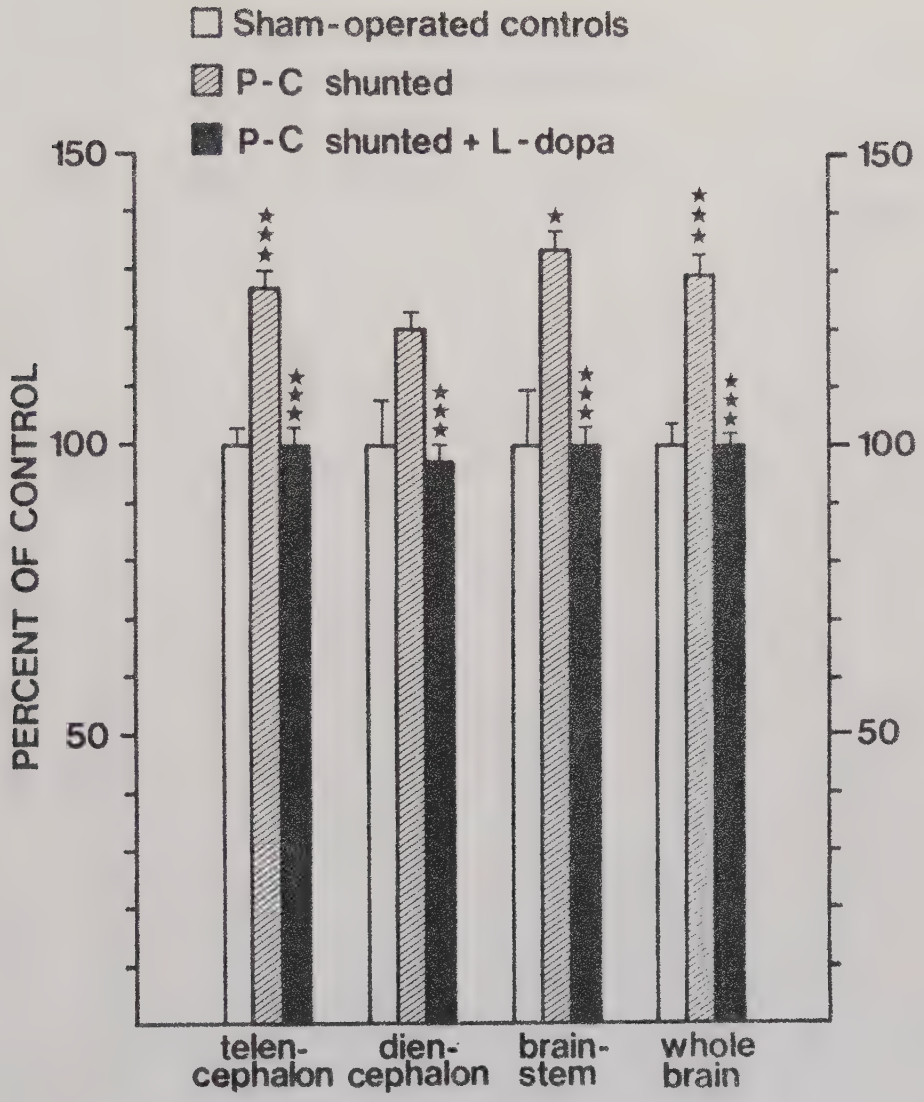
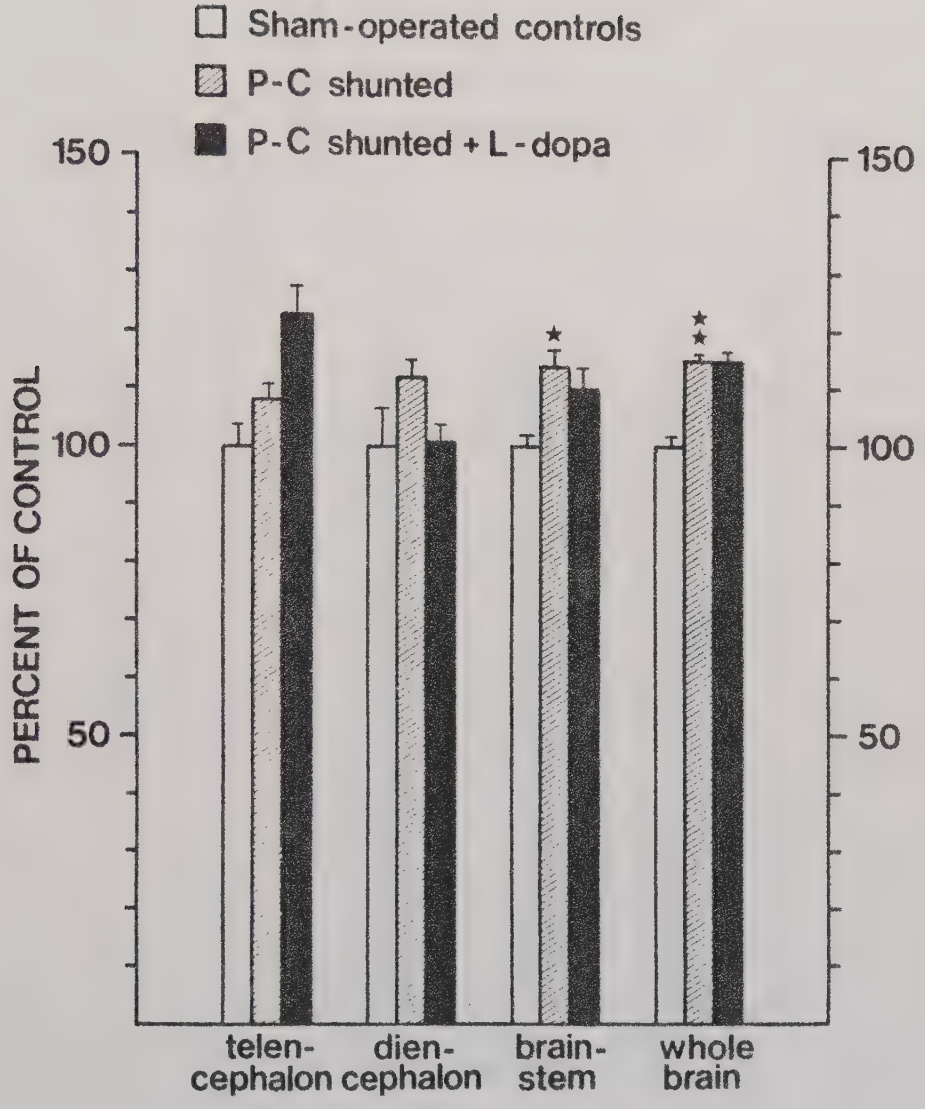

Sham-operated controls
P-C shunted
P-C shunted + L-dopa
PERCENT OF CONTROL
150
100
50
telen-cephalon
dien-cephalon
brain-stem
whole brain

FIGURE 2


Sham-operated controls
P-C shunted
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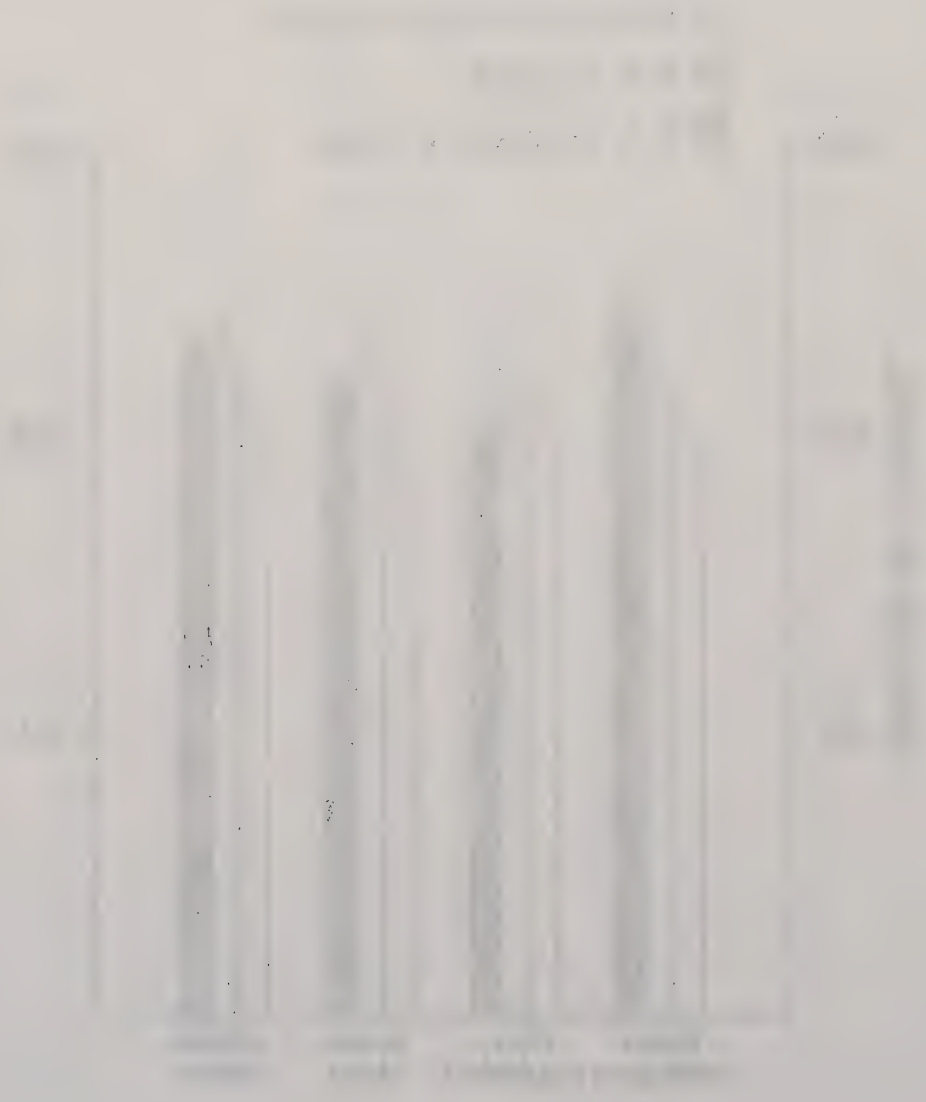
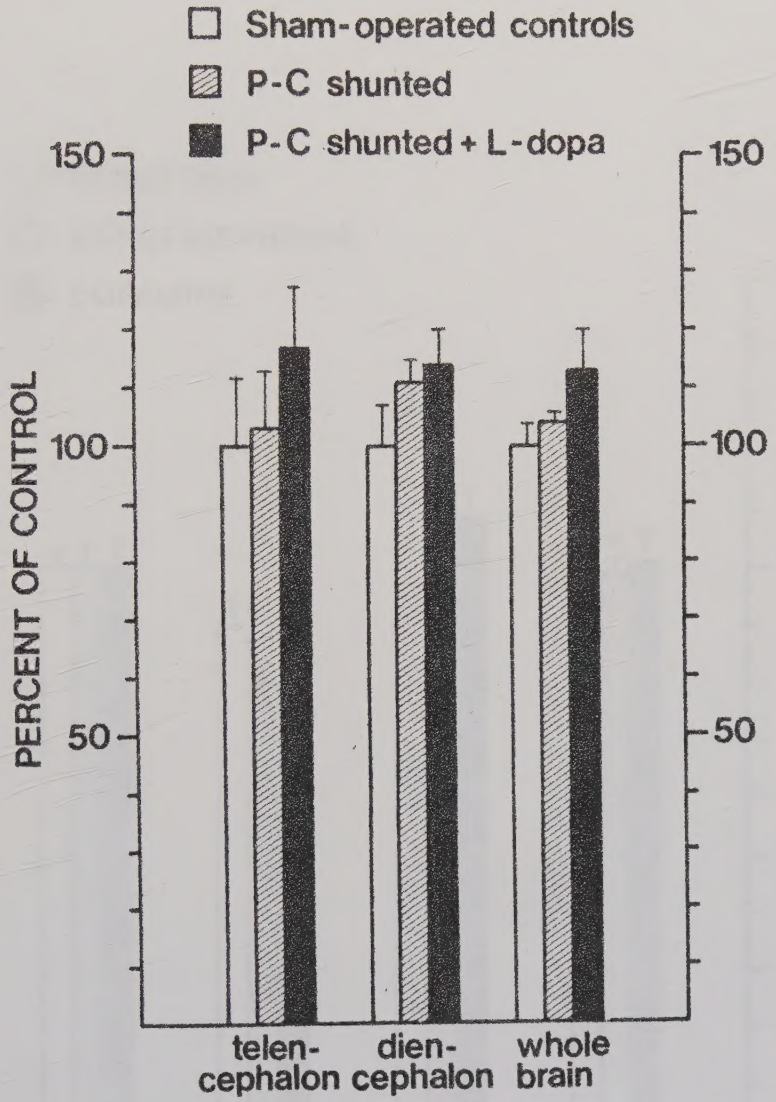
dien-cephalon
brain-stem
whole brain

FIGURE 3




Sham-operated controls
P-C shunted
P-C shunted + L-dopa
PERCENT OF CONTROL
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50
telen-cephalon
dien-cephalon
whole brain

FIGURE 4

Sham-operated controls
P-C shunted
P-C shunted + L-dopa
150
100
50
PERCENT OF CONTROL
whole brain
-dien cephalon
-telen-

FIGURE 4

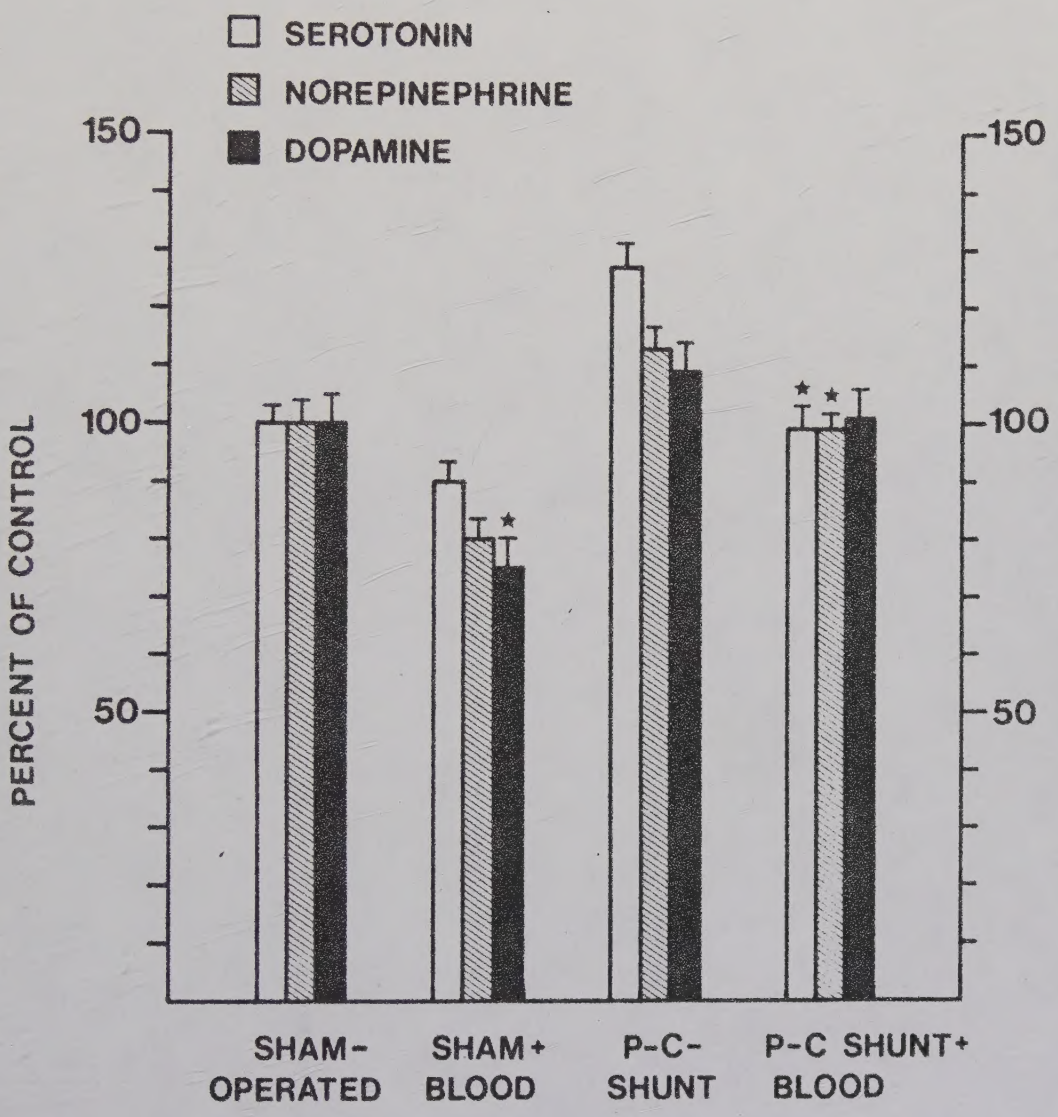
SEROTONIN
NOREPINEPHRINE
DOPAMINE
150
100
50
PERCENT OF CONTROL
SHAM-OPERATED
SHAM+ BLOOD
P-C-SHUNT
P-C SHUNT+ BLOOD

FIGURE 5

SEROTONIN
NOREPINEPHRINE
DOPAMINE
150
100
50
PERCENT OF CONTROL
SHAM-
OPERATED
SHAM+
BLOOD
P-C-
SHUNT
P-C SHUNT+
BLOOD

FIGURE 5